

THE THYROID HORMONE

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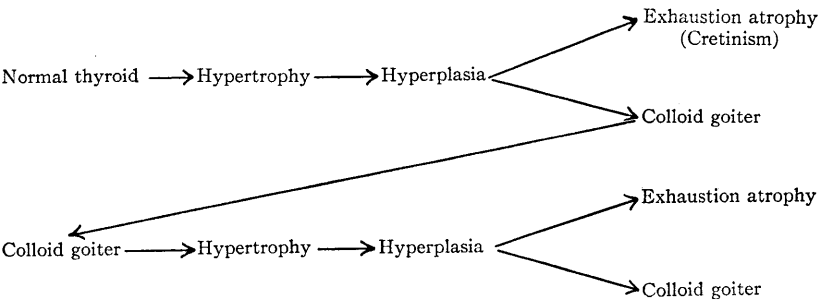
In this lecture I shall attempt to review briefly the principal facts of thyroid chemistry and physiology and some of its interrelations with other glands of internal secretion.

ANATOMY

Brief mention of the main anatomical changes will be helpful in correlating the chemical and physiological discussions which follow. *Ancestrally*, the thyroid is limited to the Chordates. In the lowest forms—tunicates, amphioxus and ammocoetes—it is represented by the endostyle organ, an elaborate midline pharyngeal epithelial groove. The connection between the endostyle organ and the ductless thyroid of all higher Chordates is established by the fortunate preservation of a single species—the lamprey—which in its larval state has an endostyle organ and in its adult form, the characteristic ductless thyroid follicles. This follicle is the thyroid unit and exists in essentially the same form in all vertebrates from the lamprey to man. It, therefore, seems safe to assume that it has essentially the same function throughout the vertebrates.

The thyroid unit is a closed rounded cyst-like vesicle of variable size (in man .05 to 0.5 mm.), lined with a single layer of low cuboidal epithelium. These epithelial cells are of two types, as first recognized by Langendorff—(1) chief cells and (2) colloid cells—and the necessary intermediate stages. These two types of cell occur in varying proportions but the chief cells in the normal gland always predominate. Because of the varying proportions of these two types of cell it is believed that they represent different stages in the secretory activity of a single cell type. The chief cell is the actively secreting cell and the colloid cell is the collapsed, spent cell. Filling the follicular spaces normally is a homogeneous amber colored globulin—the so-called colloid which gives to the thyroid its most characteristic anatomical feature. This colloid varies in density in different glands and in different follicles of the same gland and can be increased or decreased under experimentally controlled conditions which indicate that the colloid is a storage product. The

blood supply of the follicle is composed of a rich network of wide, short capillaries, very similar to that of the lung alveolus. The blood supply to the gland, as a whole, is subject to extreme variations, but even normally it ranks with the 5 or 6 most vascular organs. The thyroid is richly supplied with nerve fibers which end both in the vessel walls and around some of the gland cells. Vasomotor fibers are present in great abundance but the presence of so-called secretory or regulatory nerves is still in doubt. The fact that nerve fibers terminate in end brushes about the gland cells is evidence that they have some function, but it has been clearly demonstrated that nerve fibers are not necessary for gland function, since autotransplanted thyroid in widely separated parts of the body undergoes hyperplasia and involution simultaneously with the non-transplanted tissue and can store and excrete iodine. These manifestations of function of transplants—long known to be due to some factor reaching the cells by way of the blood stream—have in recent years been proven to be due to the thyrotropic factor of the anterior pituitary. The thyroid gland is one of the most labile tissues in the body, capable of exhibiting morphological changes within a few hours. Because of this wide range of anatomical changes we are provided with a very delicate anatomical measure of functional variations. Unfortunately attempts to interpret these changes in relation to disease have caused as much confusion as clarification. This range of morphological changes may be designated as the anatomical cycle and represented schematically as follows:



The actively hypertrophic and hyperplastic stage always indicates stimulation of the gland cells, and so far as known, this is brought about solely by the thyrotropic hormone of the anterior pituitary. The colloid stage represents the return of the hyperplastic stage to its normal or quiescent condition, and the

atrophic stage the permanently exhausted thyroid. There is no basis for assuming that the series of changes observed in the thyroid in association with simple goiter, with exophthalmic goiter, with compensatory hypertrophy following partial thyroidectomy and following the administration of anterior pituitary extracts are not essentially identical.

PHYSIOLOGY

Prior to the publication of Gull's observations (1874), "On a cretinoid state supervening in adult life in women," the function of the thyroid was either speculative—that its secretion moistened the larynx, that it served to round out the neck, that it acted as a vascular shunt for the cerebral circulation, that it aided in the formation of erythrocytes, that it neutralized toxins—or was confused with the functions of the parathyroids. Gull definitely associated atrophy of the thyroid with dryness and loss of hair, thickness and dryness of the skin and a striking deterioration of mental and physical vigor. William Ord (1878) designated this condition myxedema, because he thought the brawny thickening of the subcutaneous tissues was due to excess mucin formation. In 1882 the Reverdin brothers, and in 1883 Theodore Kocher reported more fully on the effects of total thyroidectomy in man for the cure of goiter. Their observations established the first experimental confirmation of Gull's clinical pathological observations. Kocher designated the symptom-complex *cachexia strumipriva* and recognized that it was similar to Gull's spontaneous myxedema. Following these reports many species of animals were subjected to thyroidectomy. Thyroidectomized dogs (Schiff) and cats (Smith) usually developed an acute symptom-complex (tetany) and died within a week, while rabbits, sheep and goats lived indefinitely with little or no obvious effects. No real separation of the functions of the thyroid and parathyroid was possible until E. Gley (1891), physiologically speaking, discovered the external parathyroids in the rabbit and demonstrated that the acute symptoms and fatal outcome which earlier workers had noted in man and animals following thyroidectomy was due to the removal of the parathyroids. Nearly 20 years elapsed before separate functions for the parathyroids and thyroid glands were accepted by all workers. The recent work of Aub and his coworkers indicates that there is an important interrelation between the thyroid and parathyroid as regards calcium and phosphorus metabolism.

Since 1891 many species of mammals have been subjected to thyroidectomy in which the parathyroid factor has been excluded and the results are essentially identical in all species.

The next important contribution to thyroid physiology was in 1891 when Murray cured a case of Gull's disease by a series of injections of glycerol emulsion of fresh sheep's thyroid. This was an advanced case of 5 years' duration when treatment was begun. The patient died in 1919 at the age of 74, health having been maintained for 28 years. In 1892 Howitz, Mackenzie and Fox independently demonstrated that thyroid was equally effective in curing Gull's disease when administered orally either fresh, dried or cooked.

In 1895 Magnus-Levy, using the newly developed calorimeter, discovered that in Gull's disease the heat production was lowered as much as 40 per cent. Later work showed that this lowering of metabolism (O_2 consumption, CO_2 , nonprotein nitrogen output and heat production) was the characteristic effect of thyroidectomy in all animals tested. The fall in metabolism begins in most animals between the fifth and seventh day after thyroidectomy and reaches its lowest level (30–40 per cent) in the rabbit between the 20th and 30th day and in man about the 60th day. This low level of metabolism may be maintained for years or as accessory thyroids or fragments regenerate the metabolism may rise, but usually not to the normal level.

In 1895 Magnus-Levy further showed that the oral administration of thyroid substance both to cases of Gull's disease and to normal persons caused a rise in metabolism. This observation determined the principal function of the thyroid as we understand it today, namely, that it provides a means for maintaining a higher rate of metabolism and for varying the rate to meet changing physiological needs. In 1912 Gudernatsch discovered that feeding thyroid to tadpoles caused metamorphosis to occur in from 3 to 5 days. This is still the most delicate test for the thyroid hormone. It was suggested that this effect might be due to raising the metabolism but work in recent years, particularly with the dinitrophenols, shows that these substances greatly increase the metabolism of tadpoles and cases of myxedema. This increased metabolism does not cause metamorphosis of tadpoles nor does it benefit cases of Gull's disease. The thyroid hormone, therefore, has a specific action not exhibited by iodides or by metabolism raising substances (adrenalin, dinitrophenol).

Chronologically the next important contribution to thyroid physiology was the demonstration by P. E. Smith (1916) and B. M. Allen (1917) that removal of the pituitary in tadpoles caused marked involution of the thyroid. Smith later devised the pharyngeal route for hypophysectomy in the rat and established that a hormonal factor from the anterior pituitary controlled the functional state of the thyroid.

Baumann and Hunt (1925) demonstrated that the thyroid hormone is necessary for Rubner's specific dynamic action of ingested foods. In thyroidectomized rabbits it disappears completely after 60 to 65 days, but can be restored in such rabbits by the administration of desiccated thyroid or thyroxine. While qualitatively the symptoms following thyroidectomy are similar in both immature and mature animals, the visible manifestations are much more striking in the immature animal. Superficially adult rabbits, sheep and goats may show very little change, which led the earlier workers to the conclusion that thyroidectomy was without much effect. Metabolism studies, however, showed the usual marked reduction in oxidation processes. In the young, this lowering of metabolism leads to stunting physical, mental and sexual development (cretinism).

The long life of thyroidectomized animals, the fact that animals below the lampreys do not have thyroids supports the conclusion that the gland is not essential for vegetative life.

The mode of action of thyroxine in increasing oxidation processes is unknown. The stimulation is a very general one since it increases the oxidation of proteins, fats and carbohydrates in their normal proportions as indicated by the respiratory quotient. Rohrer first showed that the oxygen consumption of minced liver, kidney and muscle of rats previously fed with desiccated thyroid was increased and Ahlgren showed that muscle from thyroidectomized animals consumed less oxygen than normal muscle. Most observations indicate that thyroxine added to excised tissue *in vitro* is without effect. The observation of Davis, de Costa and Hastings (1934) suggests that thyroxine increases the metabolism of the excised intact frog's heart but not of slices of the heart muscle. Verebely found that thyroxine was without effect on liver and muscle cells but raised the O_2 consumption of brain tissue and came to the conclusion that thyroxine probably exerts its action on the various somatic tissues by way of the central nervous system. Cannon and his coworkers found thyroxine effective in sympathectomized cats.

This conclusion is also supported by the observation of many workers including Abderhalden and Wertheimer, H. H. Meyer, Mansfield and Issekutz. Thyroxine does not raise the metabolism of frogs and reptiles. Issekutz found that thyroxine had no effect on the basal metabolism of spinal cats and assumed, as others had, that an intact central heat regulating mechanism was necessary for thyroxine action. This conception of thyroxine action would go far toward explaining its action on general metabolism, the long latent period, the relation of thyroxine to exophthalmos production, the purpose of a relatively high iodine content of the diencephalon and its possible correlation with adrenalin, that is, adrenalin acts through a peripheral sympathetic mechanism, while thyroxine may act through the vegetative centers in the hypothalamic region.

Diet. Diet influences the thyroid. Baumann and his coworkers (1896) first noted that feeding meat diets to dogs caused a decrease in the iodine store, while feeding sea fish (high in iodine) caused an increase in the iodine store of the thyroid. Watson showed that meat diets caused thyroid hypertrophy in rats. Marine and Lenhart found that liver, especially pig's liver, was the most potent of a large variety of meats in causing thyroid hypertrophy in dogs and also that this food was an important factor in the causation of goiter in brook trout. McCarrison, and later Mellanby, showed that fats also promote thyroid hypertrophy. All these studies indicate that protein and fats increase the rate of discharge of thyroxine and therefore thyroid activity is more necessary in their oxidation than in the oxidation of carbohydrates. This conclusion was also indicated by the rise in the respiratory quotient which normally follows thyroidectomy. Rachitogenic diets, whether of the high calcium and low phosphorus or high phosphorus and low calcium type, cause thyroid enlargement in dogs, rabbits and rats, providing the iodine intake is low. This action is independent of the protein, fat and carbohydrate in the diet. Such studies indicate that the ratios of calcium, magnesium and phosphorus in some important way influence thyroid function. Thompson has shown that calcium is increasingly effective in causing thyroid hyperplasia in rats the lower the iodine intake and she has recently demonstrated that a high calcium intake requires a much higher iodine intake in order to maintain a given level of the blood iodine.

BIOCHEMISTRY

Since Coindet (1820) first demonstrated the therapeutic value of iodine in the treatment of goiter many attempts to demonstrate this element in the thyroid have been made. Coindet's observation also made it possible quickly to establish that iodine was the active agent in the remedies (burnt sponge, salt, seaweed) that had been beneficially used for centuries in the treatment of goiter. The discovery by Murray (1891) of the curative effects of thyroid opotherapy in myxedema and the demonstration a year later that the active principle withstood drying and heating stimulated biochemists to attempt to isolate it. These efforts resulted in Baumann's announcement in 1895 that iodine in stable organic combination was a normal constituent of the thyroid. He obtained a brownish powder by prolonged hydrolysis with 10 per cent sulphuric acid which contained up to 10 per cent of iodine and named it *iodothyryn*, later *thyroidin*. Subsequent work showed that this method of hydrolysis destroyed part of the specific iodine compound. For some years following Baumann's discovery there was much discussion of the question whether iodine played an essential role in the active substance. Hutchinson (1896) concluded that the iodine containing colloid matter was the active substance: "The part which the iodine plays it plays in virtue of the form of combination in which it occurs in the colloid, not in virtue of its being iodine." Magnus-Levy (1897) had clearly demonstrated that iodides did not increase metabolism. Baumann and his pupils showed that iodine was present in dogs' thyroids in variable amounts and that feeding iodine increased the store, while feeding meat decreased the store. Baumann (1897) demonstrated that human thyroid glands from Hamburg contained larger amounts of iodine than glands obtained in Freiburg. Oswald (1897) demonstrated that the iodine content in general varied directly with the amount of colloid. Oswald (1899) also confirmed and extended Hutchinson's observation that the colloid was a globulin and contained practically all of the iodine. He recognized that this globulin could be rich or poor in iodine and introduced the terms "thyroglobulin" and "iodothyroglobulin." Marine and Williams (1908) and Marine and Lenhart (1909) showed that histologically the amount of colloid material in the follicle varied inversely with the degree of epithelial hypertrophy and also that the iodine content of the

thyroid varied inversely with the degree of hyperplasia. The globulin content of the thyroid is roughly the same whether the gland is hyperplastic or colloid. In the actively hyperplastic gland the thyroglobulin is in the cells and is very poor in iodine, whereas in the normal or colloid gland (goiter) the thyroglobulin is mainly in the follicles and is usually, though not always, rich in iodine. The maximum store of the iodine in the mammalian thyroid is between 5 and 6 mg. per gram of dried gland, while the minimum may be inestimable traces. The minimum concentration necessary for the maintenance of normal structure appears to be strikingly constant in the domesticated mammals and averages around one tenth of 1 per cent dry weight. The relation of the iodine store to the histological structure of a few typical mammals is given in the following table, expressed in milligrams per gram of dried gland.

	NORMAL STAGE	HYPERPLASTIC STAGE			COLLOID OR RESTING STAGE
		Early	Moderate	Marked	
Man.....	2.17	0.88	0.71	0.32	2.00
Dog.....	3.32	0.62	0.37	0.11	1.99
Sheep.....	2.47	0.40	0.01	3.00
Ox.....	3.46	1.65	0.19
Pig.....	2.51	1.10	2.35

The normal human thyroid weighs between 20 and 25 gm. and may contain as a maximum 1 mg. of iodine per gram of fresh tissue, while the average is about one half this amount. Iodine appears early in the fetal thyroid. Fenger demonstrated it in the fetal calf as early as the third month. There is also a definite seasonal variation in the iodine store (Seidell and Fenger), the store being lowest in early spring and highest in the late summer in cattle, sheep and pigs. Iodine fed to pregnant animals is quickly stored, both in the maternal and fetal thyroids and a depletion of iodine in the mother also reduces it in the fetus. Surviving thyroid in vitro has the same marked selective ability to take out and fix iodine (given as KI) from the circulating fluid as is seen in the thyroid in vivo. No other tissue has this capacity. So far as is known the thyroid is the only organ capable of elaborating the iodine containing hormone. Iodine is normally present in the blood. The normal amounts

reported vary widely from 8 to 18 micrograms per 100 cc. The variations reported are probably due more to the methods used, none of which are satisfactory, than to the actual variations, and probably 5 to 10 gamma per cent would more nearly represent the normal variations. In general the blood iodine has been found higher than normal in most cases of Graves' disease, but not in all cases and it is below normal in myxedema and after thyroidectomy (Curtis et al). Also in endemic goiter districts the blood iodine is generally lower than that of non-goiter districts. The presence of iodine in the blood implies that iodine should be present in minute amounts in all tissues as originally claimed by Justus and now generally confirmed. However, certain tissues have much higher iodine values than others, though none has quantities remotely approaching that of the thyroid which normally contains probably one fifth of the body iodine. The ovaries, anterior pituitary and diencephalon contain more iodine than other organs. The form in which the iodine exists in the blood and tissues other than the thyroid is not known. It is interesting but unprofitable at present to speculate on the possible physiological importance of the concentration of iodine in the diencephalon, ovaries and pituitary, particularly in relation to the known interrelations of these organs.

Iodine balance. Attempts have been made to determine the minimum daily needs of iodine in the normal human subject by iodine balance studies, particularly by Von Fellenberg, Scheffer, and Curtis and his coworkers. Such studies entail great difficulties because of the small amounts of iodine involved and the methods available of estimating its excretion in the urine, feces, sweat, respired air and nasal secretions. The daily requirements for thyroxine iodine can be more accurately determined by the amount of thyroxine necessary to hold the basal metabolism of cases of myxedema to the normal level. This has been determined by Boothby and other workers at 160–200 gamma I_2 (approximately 0.31 mg. thyroxine). A large part of this thyroxine in normal persons is undoubtedly derived from the conservation of iodine in the body. Hence it would be desirable to know how much iodine is required to balance that lost daily through the excreta in normal persons—so-called iodine balance. Studies along these lines have given figures of 15–25 gamma per day as the minimum intake necessary to balance excretion (v. Fellenberg, Scheffer, Curtis et al). The figures have been

obtained by determining over a period of some weeks the amount retained from a given daily intake and from the amounts excreted daily when the intake is reduced to as nearly nil as possible. The idea that there could be any constancy in the excretion of iodine under ordinary conditions seems so improbable to me that the physiological value of the data reported is problematical.

Returning to the attempts to isolate the active principle of the thyroid, all efforts to utilize the method of acid hydrolysis failed. Oswald tried barium hydroxide hydrolysis because Drechsel (1896) had used this method successfully in isolating iodogorgin from the axial skeleton of coral. Wheeler and Jamieson (1905) showed iodogorgin 3.5.3¹.5¹. was diiodotyrosine. Oswald failed to obtain an active fraction because, as pointed out later by Harington, he discarded the acid insoluble portion with the barium sulphate. Hutchinson, Oswald and others, also were unsuccessful, in concentrating the active principle by means of peptic and tryptic digestion. Kendall (1913) again undertook to use the method of alkaline hydrolysis and showed that the iodine became increasingly dialyzable through celloidin membranes by carefully treating thyroid with sodium hydroxide. In 1914 Kendall, by hydrolyzing thyroid with 1 per cent sodium hydroxide in 90 per cent alcohol, was able to separate the iodine of the thyroid into two fractions which he designated A and B. Fraction A, or the acid insoluble, was physiologically active, while fraction B, or the acid soluble portion, was physiologically inactive. In 1916 Kendall announced the isolation from fraction A of a crystalline compound containing approximately 65 per cent of iodine and which physiologically produced the same effects as desiccated thyroid. The yields were exceedingly poor and his attempts to determine the structure from amounts available were unsuccessful but he thought it was a derivative of tryptophane. Nurnberg (1907) using Baumann's iodothyrim attempted to ascertain the iodine holding group of thyroglobulin. He was unsuccessful but certain reactions led him to suggest that the iodine was bound with tyrosine—probably as diiodotyrosine. In 1926 Harington improved the method of extracting thyroxine by returning to the use of barium hydroxide and was able to accumulate sufficient quantities to determine its empirical formula as $C_{15}H_{11}O_4NI_4$ and its structural formula as 3.5.3¹.5¹. tetraido-4-(4¹-hydroxyphenoxy) phenyl α amino-propionic acid. The organic nucleus he named thyronine.

Later work by Harington and Randall showed that thyronine was formed from two molecules of tyrosine with the loss of one molecule of a aminopropionic acid and the two benzene rings joined by an ether linkage. In 1927 Harington and Barger synthesized thyroxine and showed that it had the same physiological action as natural thyroxine. Harington further showed that the acid soluble fraction, or the physiologically inactive iodine, earlier designated product B by Kendall, was diiodotyrosine and identical with that present in coral and sponge. Foster (1929) confirmed the occurrence of diiodotyrosine in the thyroid and was able to recover as much as 30 per cent of the total thyroid iodine as diiodotyrosine. It is of paleochemical interest that diiodotyrosine in protein combination is found in sponge and coral and may be the hormonal equivalent of thyroxine in these animals. Harington has estimated that diiodotyrosine on the average accounts for about 60 per cent of the thyroid iodine and thyroxine for 40 per cent. The proportion of these two compounds must obviously vary since diiodotyrosine is a stage in the synthesis of thyroxine and both substances are constituent amino acids of a specific globulin. These are the only two forms in which iodine has been found in the thyroid.

It is of interest to note that one may immunize an animal (rabbit) against ox iodothyroglobulin without affecting the physiological action of the hormone (Rosen and Marine) while with other hormones of a protein nature, such as parathormone, the anterior pituitary hormones and prolactin appear to be, immunity eliminates their physiological activity. This shows that thyroxine is not an essential factor in the antigenic property of thyroglobulin while with the other hormones mentioned their antigenic properties are intimately connected with the physiologically active component of the protein. The synthesis of thyroxine by the thyroid requires hours while the storage of iodine in the surviving gland, both in vivo and vitro, is almost instantaneous. The form in which iodine is so quickly stored has not been determined but the work of Harington suggests that it may be bound with tyrosin. It is, however, firmly fixed so that prolonged perfusion will not wash it out if the cells are surviving. This is not true of other tissues. The first evidence of thyroxine formation in dogs' thyroid using the Gudernatsch tadpole test was obtained by Marine and Rogoff in about 8 hours after the intravenous injection of potassium iodide. It

has been known since thyroxine was first available that when orally administered it is much less active than an equivalent amount of iodine in the form of desiccated thyroid or of iodothyroglobulin. This has been explained as due to its insolubility and hence its poor absorption. But Reed Hunt (1923) showed that when thyroxine was injected intravenously and iodine equivalent amounts of desiccated thyroid administered orally thyroxine was less active, thus establishing that the total iodine of thyroglobulin (thyroxine and diiodotyrosine) was physiologically active. Recently this fact has been confirmed in another way. Rihl, Oestreicher and Reiss (1936) showed that in the rabbit the pulse rate rises almost immediately after the injection of thyrotropic hormone, whereas when thyroxine is injected no effect on pulse rate is noted for more than 6 hours.

Salter and Lerner have suggested that diiodotyrosine is a cohormone and the thyroid hormone is complete only when diiodotyrosine and thyroxine are combined in the peptone molecule. In support of their hypothesis they have taken the relatively inactive (as tested on cases of myxedema) diiodotyrosine peptone residue from human thyroglobulin after removing most of the thyroxine and with the aid of pepsin have resynthesized a protein that was as effective in relieving myxedema as natural thyroglobulin in iodine equivalent doses. No explanation of what has happened under these conditions is available and the phenomenon is at present limited to the degradation products of thyroglobulin. Recently Ludwig and von Mutzenbecher have produced a substance chemically and physiologically nearly identical with thyroxine from a "non-specific protein" and iodine by hydrolyzing with weak alkali and heat. Heretofore only diiodotyrosine has been obtained by hydrolyzing non-specific protein plus iodine. Diiodotyrosine has only the action of iodides. It does not enhance the activity of thyroxine. Harington has combined the two amino acids into thyroxyl-diiodotyrosine and diiodotyrosyl-thyroxine without influencing the activity of contained thyroxine.

As the matter stands at present there is no need of assuming the existence in the thyroid of any active compounds other than those determined by iodine and thyronine. The combinations of iodine and thyronine are not the active principles but rather they are the components that confer specific properties upon a more or less complex protein fragment. This opinion is supported by the work of Harington and of Salter and his coworkers

who have found thyroxine peptone more active than glycyl or allanyl thyroxine and these in turn somewhat more active than thyroxine in experiments where all three groups of substances were given intravenously. Much work obviously remains to be done to determine why thyroxine and diiodotyrosine combined in thyroglobulin is so much more active physiologically than the equivalent amount of crystalline thyroxine and diiodotyrosine.

INTERRELATIONS

This is probably the most important and certainly the most difficult problem in the thyroid physiology and pathology.

Thyroid-Gonads. This is the oldest known interrelationship of the thyroid. It is evidenced by the frequency of enlargement of the gland during menstruation, pregnancy, puberty and the menopause. As has already been mentioned, the ovary (but not testis) is relatively high in iodine, but the form in which it occurs and its possible function are wholly unknown. Removal of the gonads in the rabbit, dog, and rat usually leads to a slow involution of the thyroid, becoming manifest in about one month and associated with a slight reduction in total metabolism. In the guinea pig, on the other hand, gonadectomy causes a definite slight temporary activation and enlargement of the thyroid. Schockaert noted that emulsions of fresh ox anterior pituitary caused greater hypertrophy of the accessory male sex glands after thyroidectomy than before. I have pointed out that thyroidectomy in puberal rabbits hastens sexual maturation and greatly increases sexual activity in the males. Both of these types of reaction are apparently mediated through the anterior pituitary, as is further indicated by the fact that administration of oestrogenic substances or aqueous extracts of the male germinal epithelium prevent the thyroid enlargement in castrated guinea pigs, while desiccated thyroid prevents increased sexual activity of thyroidectomized male rabbits.

Thyroid-Thymus. Gudernatsch (1914) observed that thymus feeding increased the rate of growth of tadpoles, definitely retarded their metamorphosis and offered some protection to them against thyroid feeding. Hoskins obtained increase in the weight of the fetal thymus in guinea pigs whose mothers had been fed desiccated thyroid. Thyroidectomy definitely hastens thymus involution. Removal of the pituitary in dogs (Houssay) usually causes involution of the thymus. In diseases in which

the thyroid activity is increased, as in exophthalmic goiter and acromegaly, thymus hypertrophy frequently occurs. All these effects could be interpreted as indicating some kind of an antagonism between thymus and thyroid hormones directly, or that they were mediated through the pituitary.

Thyroid-Parathyroids. The view that the parathyroids could function vicariously for the thyroid ended with Gley's demonstration that the so-called acute effects of thyroidectomy were due to the accompanying parathyroidectomy. The strikingly different symptoms of thyroidectomy and parathyroidectomy appeared to establish independent functions for these glands, but during the last decade evidence has accumulated indicating that they are at least complimentary in certain of their functions. The work on experimental rickets and particularly the work of Aub and his coworkers on exophthalmic goiter, thyroid feeding and myxedema shows that the thyroid is also concerned with the metabolism of calcium and phosphorus. The idea that excessive calcium ingestion was a causal factor in goiter was suggested a century ago (McClellan 1837). Aub has shown that in Graves' disease the calcium excretion is greatly increased while in myxedema it is lessened. Both thyroid feeding and the administration of the thyrotropic hormone greatly increase calcium excretion (Pugsley). Excessive thyroid feeding causes parathyroid hypertrophy. The increased thyroid activity in animals (dogs, rats, rabbits) on rachitogenic diets is associated with parathyroid hypertrophy. Thyroidectomy in rabbits on diets with normal Ca:P shows involution of the parathyroids. Baumann (unpublished) has shown that thyroidectomized rabbits on a high phosphorus and low calcium diet die more quickly than normals—suggesting that the thyroid enlargement on rachitogenic diets is protective in nature.

Thyroid-Chromaffin System. Some interrelation is indicated because of the facts that thyroxine and epinephrine are both derivatives of tyrosin and that both are powerful activators of metabolism through different mechanisms. Also as Goldberg and others have noted in thyroidectomized lambs—and we have confirmed in thyroidectomized rabbits—the adrenal medulla is definitely hypertrophic.

Asher and Flack (1911) showed that the blood pressure response in rabbits to a given dose of adrenalin was greater after stimulation of the thyroid nerve with intact thyroid than before. This has been confirmed, particularly by Cannon and his

coworkers. Oswald has shown that a similar increase in the blood pressure response to adrenalin may be obtained by the intravenous injection of iodothyroglobulin and that the effect produced is proportional to the iodine content of the thyroglobulin injection. The Goetsch epinephrine test on exophthalmic goiter may be a clinical application of increased sensitivity to epinephrine. All these observations would support the original view of Asher and Flack that the thyroid hormone increases the irritability of the sympathetic nervous system and sensitizes the tissues innervated by it so that they are more susceptible to stimulation by epinephrine.

Thyroid-Pituitary. It has been known for nearly a century that individuals and animals with simple goiter have greatly enlarged anterior pituitaries. Niepce observed pituitaries in goitrous cretins weighing 2.4 gm. Rogowitsch (1889) was the first to produce experimentally hypertrophy of the anterior pituitary by removal of the thyroid in rabbits. This has been generally confirmed and a generalization may be made that in all states of thyroid insufficiency the anterior pituitary tends to undergo hypertrophy. Histologically all the elements of the anterior pituitary appear to take part in this hypertrophy, but the most striking single feature in rabbits is the degranulation of the acidophilic cells. The physiological significance of this thyroid-pituitary relationship became evident only after Allen and Smith devised methods for removing the pituitary in tadpoles and later by Smith developing a similar method for rats. Smith noted in tadpoles and in rats that hypophysectomy caused a marked reduction in the volume of the thyroid gland and that implants or injections of extracts of fresh anterior pituitary restored such atrophic thyroids to normal and even produced hypertrophy. Loeb, Aron, Schockaert, Uhlenhuth and many others have confirmed and extended this observation, using emulsions, acid and alkaline extracts of fresh and dried anterior pituitary. Eitel, Krebs and Loeser have also shown that anterior pituitary extract will cause hypertrophy of surviving thyroid cells in vitro. Marked hyperplasia of the thyroid, beginning within a few hours, occurs in susceptible animals. This is associated with a rapid loss of iodine from the thyroid and increase in the blood iodine, increase in metabolic rate and increased excretion of calcium and creatin. Exophthalmos also may develop in susceptible species (birds, guinea pigs). It was early pointed out that this symptom complex was strikingly

similar to that seen in exophthalmic goiter. This thyroid stimulating substance is one of several specific hormones of the anterior pituitary (adrenotropic, growth promoting, lactogenic, gonadotropic and possibly splenotropic, parathyrotropic and thymotropic).

The thyrotropic factor has been partially separated from the others by Collip and his coworkers and the chemical and immunological data, while still meagre, indicate that the active substance is probably protein in nature. Sufficient work has now been done with the thyrotropic factor to justify the conclusion that the thyroid may be directly stimulated only by it, irrespective of whether the thyroid stimulation is associated with endemic goiter, with Graves' disease, with compensatory hypertrophy following partial thyroidectomy or with gonadectomy. While the anterior pituitary, therefore, exercises a controlling influence on the thyroid there is evidence that the thyroid exercises equally as profound an influence on the pituitary. Thus the anatomical changes in the anterior pituitary following thyroidectomy can be entirely prevented and cured by giving desiccated thyroid or thyroxine. Studies on the interrelations of the thyroid-pituitary have not appreciably advanced our knowledge as to the cause of myxedema or endemic goiter. The atrophy of the thyroid in myxedema is associated with enlargement of and increase in the thyroid stimulating factor of the pituitary, while in Graves' disease neither pituitary enlargement nor increase in the thyrotropic factor can be demonstrated. The relation of the thyroid secretion to the development of the exophthalmos of exophthalmic goiter has long been a controversial question. Prevailing opinion still favors the view that it is in some way connected with a hypersecretion of the thyroid hormone.

Without going into details regarding the mechanism by which chronic progressive exophthalmos is produced, one may say that in the lower animals the neuromuscular mechanism discovered by Mueller (1858) has not been disputed. In man and in monkeys, which have complete bony orbits and vestigial peri-orbital muscles, there must be the additional factor of weakness of the recti muscles. The first published work on the experimental production of exophthalmos was by Schockaert in young ducks and in the same year by Leo Loeb in guinea pigs following the injection of anterior pituitary extracts and muscles. About this time we reported the occurrence of progressive

exophthalmos in rabbits which developed goiter on a diet of alfalfa hay and oats plus daily injections of acetonitrile. Assuming that the goiter was a manifestation of thyroid insufficiency and that exophthalmos was in some way connected with this insufficiency, we tried thyroidectomy and found that this produced exophthalmos much more quickly. We then tried the effect of pituitary extracts in normal and thyroidectomized guinea pigs and also found that exophthalmos was more easily produced after thyroidectomy than before. The importance of a relative or absolute thyroid insufficiency as one of the factors in the development of exophthalmos seems thoroughly established. Likewise the factor causing the exophthalmos must be present in the anterior pituitary, yet it does not occur in myxedema and cretinism, although both thyroid insufficiency and pituitary hyperactivity are present. Obviously other factors are involved. In the rabbit, and in man too, sex plays a rôle. It is much more frequent in the males, and gonadectomy in the rabbit both lessens the incidence and causes a regression of an existing exophthalmos. It is possible that oestrin is the factor that protects the female by preventing pituitary overactivity. Thyroid therapy is effective both in preventing and in curing this form of exophthalmos in rabbits and guinea pigs (and to some extent in human exophthalmos). Exophthalmos cannot be produced after the sympathetic pathway to the eye has been divided (Horner's syndrome) which indicates that it is dependent upon the stimulation of a sympathetic center in the central nervous system—probably the hypothalamus. The high iodine content of this portion of the brain may be significant in this connection. Thus exophthalmos would appear to be dependent upon an increased irritability of the sympathetic center which is determined by the concentration of thyroxine, thyrotropic hormone, certain salts (Ca, Mg, P) and probably other hormones bathing it. If this should be shown to be the mechanism of exophthalmos production it would be an additional factor in favor of the central action of thyroxine as well as the demonstration of another important thyroid pituitary interrelation.

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